VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 4, Number 1, 2004

Research Paper

Host Feeding Patterns of Established and Potential Mosquito Vectors of West Nile Virus in the Eastern United States

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ABSTRACT

An important variable in determining the vectorial capacity of mosquito species for arthropod-borne infections is the degree of contact of the vector and the vertebrate reservoir. This parameter can be estimated by examining the host-feeding habits of vectors. Serological and polymerase chain reaction based methods have been used to study the host-feedings patterns of 21 mosquito species from New York, New Jersey, and Tennessee, 19 of which previously have been found infected with West Nile virus. Mammalophilic mosquito species in New Jersey and New York fed primarily upon white-tailed deer, while those from Memphis, Tennessee, fed mainly upon domestic dogs. A total of 24 different avian host species were detected among the avian-derived blood meals. American Robin, Northern Cardinal, Northern Mockingbird, Tufted Titmouse, and Brown-headed Cowbird were common avian hosts, while blood meals derived from the American Crow were relatively rare. Although the majority of common host species were potentially among the most abundant birds at each location, the proportion of blood meals from the most commonly fed upon avian species was greater than was predicted based upon the likely abundance of these species alone. These findings suggest that vector species for West Nile virus may preferentially feed upon certain avian hosts. Key Words: Arbovirus—Mosquito—Host—Vector blood meal. Vector-Borne Zoonotic Dis. 4, 71–82.

INTRODUCTION

SINCE ITS INTRODUCTION IN 1999, West Nile Virus (WNV) has become a significant public health problem in the United States, with more than 4,000 human cases reported in 2002 (Centers for Disease Control and Prevention 2003b). WNV is a flavivirus, and is related to Saint Louis encephalitis virus (SLE), a viral infection also endemic to the United States. Both

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SLE and WNV are primarily infections of wild birds and are transmitted by mosquitoes. The viruses are maintained in avifauna primarily through a cycle involving ornithophilic mosquitoes (Campbell et al. 2002). Mammals, including humans, are dead end hosts that are infected by mosquitoes with catholic feeding habits that feed on both mammals and birds.

WNV has been detected in 43 different mosquito species (Centers for Disease Control and Prevention 2003c). However, *Culex* (*Culex*), and in particular *Culex pipiens* L., *Cx. quinquefasciatus* Say, *Cx. salinarius* Coquillett, and *Cx. tarsalis* Coquillett appear to be the most important vectors of WNV in North America, based on the frequent detection of WNV in these species (Andreadis et al. 2001, Bernard et al. 2001, Blackmore et al. 2003), and their laboratory vector competence (Sardelis et al. 2001, Dohm et al. 2002, Goddard et al. 2002). Although these *Culex* species are ornithophilic (Tempelis 1975, Magnarelli 1977, Irby and Apperson 1988), in keeping with the predicted feeding behavior for an enzootic vector, some studies have reported that a proportion of their meals are taken from mammals (Edman 1974, Zimmerman et al. 1985, Irby and Apperson 1988, Apperson et al. 2002), suggesting that these mosquitoes may be capable of transmitting WNV to mammals as well as maintaining the avian enzootic transmission cycle (Savage et al. 1999).

An important variable in the ecology of WNV transmission is the degree of bird-vector contact. The simplest hypothesis is that mosquitoes do not discriminate among bird species. If so, the proportion of blood meals derived from each bird species would reflect their relative abundance. Alternatively, certain bird species might be preferentially fed upon based on physiological, behavioral or ecological factors that make one species more attractive or available than others to vector mosquitoes. Such differences would play in important role in determining which avian species are reservoirs for perpetuating WNV epidemics.

In the present study, we used ELISA and PCR-based methods to identify the vertebrate hosts of blood meals from over 1,700 mosquitoes collected from three geographic locations in the northeastern and South Central United States in 2001. WNV transmission was ongoing in all three areas during the summer of 2001. Our objectives were to characterize host-feeding patterns of potential vector mosquitoes for WNV, with particular emphasis on identifying avian hosts of *Culex* mosquitoes, as a means of determining if certain bird species appeared to be preferentially targeted.

MATERIALS AND METHODS

Collection of blood-fed mosquitoes

Blood-fed mosquitoes were collected throughout New Jersey between May and October 2001. Five pre-existing Eastern Equine Encephalomyelitis (EEE) surveillance locations (Centerton, Salem County; Corbin City, Atlantic County; Dennisville, Cape May County; Green Bank, Burlington County; Waterford, Camden County) were sampled once a week using resting boxes. These sites were situated in dense pine plantations that are in the vicinity of permanent freshwater swamps (red maple/white cedar). Resting mosquitoes were also collected weekly from a historic abandoned structure (Fort Mott) near Delaware Bay in Salem County. This site is located in a more sparsely populated rural setting bordered by salt marshes and dredge spoils. All of these sites were located in Southern New Jersey, roughly 150 miles south of New York City. An additional weekly resting box location was added in Tenafly, Bergen County (in Northern New Jersey). This site was surrounded by a more suburban environment, but was also situated near a red maple freshwater swamp.

Mosquitoes were collected at 10 sites in Westchester County (north of New York City) from June through September. All sites were located within hardwood forests dominated by oak, hickory, and maple trees. Dominant understory included fern, nettle, grasses, and barberry.

Mosquitoes were also collected in Memphis/Shelby County, Tennessee, within and surrounding the city of Memphis. Mosquitoes were collected from natural resting sites in concrete and galvanized metal storm water sewers.

Identification of mosquitoes

Mosquitoes were sorted on a chill table and identified using morphological characters (Apperson et al. 2002), placed individually into labeled cryotubes and stored at −70°C. *Cx. pipiens, Cx. quinquefasciatus* and hybrids occur together in the Memphis area and therefore specimens of these taxa were referred to as *Cx. pipiens* complex (Jakob et al. 1979, Aspen and Savage 2003). Specimens of *Anopheles quadrimaculatus,* due to their rubbed condition upon receipt, were initially identified morphologically as *Anopheles quadrimaculatus sensu lato.* These were subjected to PCR for species identification (Rafferty et al. 2002). Of 438 samples analyzed, 428 produced products of a size expected for *A. quadrimaculatus sensu strictu*. No evidence was seen of any other member of the complex.

A representative subsample of specimens identified morphologically as *Cx. pipiens, Cx. pipiens complex, Cx. restuans* and *Cx. salinarius* were analyzed by PCR for molecular identification. Taxon-specific PCR primers for *Cx. restuans, Cx. salinarius* and *Cx. pipiens* complex were employed for this purpose, as previously described (Aspen et al. 2003, Aspen and Savage 2003).

Blood meal identification

Blood fed mosquitoes were initially processed for blood meal identification by indirect ELISA as previously described (Irby and Apperson 1988). Abdomens of blood fed mosquitoes were homogenized in 500 μL of phosphate buffered saline (pH 7.4) and the homogenate subjected to centrifugation at 6225 × g for 5 min. A total of 400 μL of the supernatant was removed for use in the ELISA. The remaining solution was brought to 10 mM EDTA, the pelleted material resuspended and the samples stored at −80°C. The supernatants were initially classified using a panel of broadly reactive antisera (anti–mammal, reptile, avian, and amphibian) as previously described (Irby and Apperson 1988). Extracts positive for mammalian blood were further characterized using a panel of species-specific antisera.

Samples testing positive for avian blood were further classified by PCR. DNA was extracted from 50 μL of archived samples testing positive for avian blood, a portion of the cytochrome B gene from the avian derived blood meals amplified as peviously described (Apperson et al. 2002). Two aliquots of the resulting PCR product were then separately mixed with 6 μL of two PCR product drivers derived from Northern Cardinal or Carolina Chickadee. Heteroduplex formation and separation of the heteroduplex products were carried out as previously described (Lee et al. 2002). Samples were identified on the basis of a comparison of the relative mobility of the HDA bands to those of standard samples derived from avian blood DNA. PCR products producing HDA patterns that did not match any standard were subjected to DNA sequence analysis and their identity determined by comparison to the Genbank DNA sequence database.

Nominal 95% confidence intervals were calculated for all collections in which the number of individual blood meals identified exceeded 20. Confidence intervals were calculated using the formula 95% CI = $\pm 1.96 \times$ (square root $p(1 - p)/n$), where p = the proportion of blood meals from a given source, and n = the total number of blood meals identified (Steel et al. 1997).

Avian abundance data

Abundance data for birds at each location were obtained from the North American Breeding Bird Survey (Sauer et al. 2003). The North American Breeding Bird Survey collects data from roadside surveys conducted in June of each year. Roughly 2,900 24.5-mile long routes are surveyed in the Continental United States every year, providing a fairly detailed map of avian species abundance (Sauer et al. 2003). For the purposes of this study, all roadside routes within a 25-mile radius of each mosquito collection point were identified. Bird abundance data from the survey routes within the 25-mile radius of each collection point were pooled to provide an estimate of the abundance of the avifauna at each site. Data from 10 routes were analyzed from New Jersey, three routes from

Westchester County, and six routes from Memphis/Shelby County, Tennessee.

RESULTS

Mosquito species identification

All of the mosquitoes were identified using a combination of morphological and molecular criteria, as described above. To confirm the morphological identifications, 161 *Cx.* (*Culex*) mosquitoes (73 from New Jersey, 16 from New York, and 72 from Tennessee) were randomly selected and tested by molecular procedures (Aspen et al. 2003, Aspen and Savage 2003). PCR products were obtained for 146 (91%) of samples tested. Morphological and molecular identification corresponded exactly in 93% (136/146) of the specimens. Molecular identification resulted in refined identifications in 2% (3/146) of specimens, in which morphological identifications of *Cx. restuans/pipiens* complex were resolved to the correct species. Seven errors (5%) in morphological identification were detected: five specimens of *Cx. restuans* were identified morphologically as *Cx. pipiens* complex and two specimens of *Cx. pipiens* complex were identified as *Cx. restuans*. All 12 (100%) specimens of *Cx. salinarius* examined were correctly identified.

Blood meal identification

A total of 1,735 blooded mosquitoes representing 21 different species were included in the study (Table 1). The two most frequently collected species (*An. quadrimaculatus s.s.* and *Cx. pipiens* complex) together represented roughly 60% of the blooded mosquitoes collected. Of 1,735 meals examined, 1,540 (89%) were successfully identified to the class level. Mammals were found to represent the most common host class (Table 1). Several species, including *Anopheles quadrimaculatus s.s.*, *Ae. vexans* Meigen, *Ochlerotatus japonicus* (Theobald) and *Oc. trivittatus* (Coquillett), fed exclusively or almost exclusively on mammals. Mammalian-derived blood meals were further classified to species level. A total of 1,028/1,144 mammalian-derived blood meals examined (90%) were identified to the species level. Ten different mammalian hosts were found in the blood meals examined (Table 2). In New York and New Jersey mosquitoes most frequently fed upon white-tailed deer (*Odocoileus virginianus*). In contrast, in Memphis, Tennessee, dogs were the principal mammalian hosts, representing 74% of mammalian-derived meals.

Four species (*Cs. melanura* Coquillett, *Cx. pipiens* complex, *Cx. restuans* and *Cx. salinarius* Coquillett) frequently contained avian-derived blood meals. Of these, *Cs. melanura* was the most ornithophilic species, with 87.5% of the blood meals taken from avian hosts. The proportion of avian-derived blood meals detected in *Cx. pipiens* and *Cx. pipiens* complex mosquitoes varied by location, ranging from 34.7 ± 7.6% in New Jersey, to 84.6 ± 19.6% in New York.

Avian-derived blood meals were classified to the species level by PCR-HDA (Lee et al. 2002) (Table 3). Of 213 avian-derived meals, 137 (64%) produced analyzable HDA patterns. The majority of the samples that were not identifiable did not produce a detectable amplification product. A total of 24 different interpretable PCR-HDA patterns were found to exist among the 137 samples, while 4 (3%) of the samples gave HDA patterns containing greater than two bands, indicative of a meal containing the blood of two or more avian species. Of these 24 different groups, 18 could be definitively identified (Table 3). The remaining six groups contained DNA sequences that, while clearly avian in nature, did not exactly match any of the DNA sequences currently in the Genbank database. These are marked as "unknown" and the species whose sequence was most similar to that of the unidentified group identified in the footnote.

Three different collections (i.e., single species collected at a particular location) contained 20 or more identifiable avian-derived blood meals (Fig. 1). Within each of these collections, the three most common avian hosts together represented over 60% of the blood meals identified (Fig. 1). The Northern Cardinal was among the three most common hosts in all three collections. The American Robin was one of the most common hosts in both col-

TABLE 1. PROPORTION OF BLOOD MEALS TAKEN FROM DIFFERENT HOST CLASSES

		Percentage feeding on[a]			
	ID/tested	Amphibian	Bird	Mammal	Reptile
New Jersey					
Ae. vexans	8/8	12.5	0.0	87.5 ± 22.9	0.0
An. bradleyi	41/41	0.0	2.4 ± 4.7	97.6 ± 4.7	0.0
An. crucians/bradleyi	50/50	0.0	8.0 ± 7.5	92.0 ± 7.5	0.0
An. punctipennis	20/20	5.0 ± 9.6	15.0 ± 15.6	70.0 ± 20.1	10.0 ± 13.1
An. quadrimaculatus	415/416	0.2 ± 0.5	1.2 ± 1.0	97.6 ± 1.5	1.0 ± 0.9
Cs. melanura	68/68	0.0	89.7 ± 7.2	10.3 ± 7.2	0.0
Cx. pipiens	150/190	17.3 ± 6.0	34.7 ± 7.6	38.0 ± 7.8	10.0 ± 4.8
Cx. restuans	25/29	8.0 ± 10.6	52.0 ± 19.6	32.0 ± 18.3	8.0 ± 10.6
Cx. salinarius	57/58	3.5 ± 4.8	24.6 ± 11.2	71.9 ± 11.7	0.0
Oc. sollicitans	15/15	0.0	0.0	100.0	0.0
Oc. thibaulti	9/9	0.0	0.0	100.0	0.0
Oc. triseriatus	6/6	0.0	50.0	50.0	0.0
New York					
Ae. cinereus	30/30	0.0	0.0	100.0	0.0
Ae. vexans	40/43	0.0	0.0	100.0	0.0
An. punctipennis	6/6	0.0	0.0	100.0	0.0
Cq. perturbans	29/42	0.0	3.4 ± 6.6	96.6 ± 6.6	0.0
Cs. melanura	4/6	0.0	50.0	50.0	0.0
Cx. pipiens	13/19	7.7	84.6	0.0	7.7
Cx. restuans	10/10	0.0	80.0	20.0	0.0
Cx. territans	16/56	75.0	12.5	0.0	12.5
Oc. canadensis	5/5	0.0	0.0	100.0	0.0
Oc. japonicus	53/54	0.0	0.0	100.0	0.0
Oc. taeniorhynchus	8/8	0.0	0.0	100.0	0.0
Oc. triseriatus	31/31	0.0	0.0	100.0	0.0
Oc. trivittatus	47/47	0.0	0.0	100.0	0.0
Ps. ferox	5/5	0.0	0.0	100.0	0.0
Tennessee					
An. quadrimaculatus	169/180	2.4 ± 2.3	0.6 ± 1.2	96.4 ± 2.8	0.6 ± 1.2
Cx. erraticus	50/54	2.0 ± 3.9	10.0 ± 8.3	86.0 ± 9.6	2.0 ± 3.9
Cx. pipiens complex	154/222	2.6 ± 2.5	71.4 ± 7.1	24.0 ± 6.7	1.9 ± 2.2
Cx. territans	6/7	83.3	0.0	16.7	0.0

[a]Only species for which the number of blood meals identified was 5 or greater are shown. The first column indicates the number of mosquitoes identified and the overall number tested. All other columns indicate percentages (±95% CI when applicable) of meals from a given host class identified in a given mosquito species. 95% confidence intervals are provided for species where the number of samples identified was greater than or equal to 20.

lections from New Jersey, while the Northern Mockingbird was the most common host in *Cx. pipiens* complex mosquitoes from Tennessee.

The abundance of different bird species at each location was estimated from data collected by the North American Breeding Bird Survey (Table 4). In almost all cases, the most commonly fed upon birds were among the 20 most abundant avian species identified. The only exceptions to this were in New York, where the Wood Thrush (22nd in abundance) and Ring-necked Pheasant (52nd in abundance) were among the top three hosts.

DISCUSSION

Most arboviral encephalitides endemic to the United States follow a similar life history. The virus is initially maintained in an enzootic cycle involving the local avian fauna and ornithophilic mosquitoes. As the infection intensifies, the virus becomes more available to catholic feeding mosquito species, mediating transmission of the virus to mammalian hosts. WNV is apparently following a similar ecological pattern in the United States, primarily becoming an infection of local avifauna. In the studies reported here, we have examined the

TABLE 2. ANALYSIS OF MAMMALIAN BLOOD MEAL SOURCES

		Percentage feeding on[a]									
	ID/tested	*Cat*	*Deer*	*Dog*	*Horse*	*Human*	*Pig*	*Opossum*	*Rabbit*	*Raccoon*	*Squirrel*
New Jersey											
Ae. vexans	6/7	0.0	66.7	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0
An. bradleyi	38/40	0.0	84.2 ± 11.6	2.6 ± 5.1	2.6 ± 5.1	2.6 ± 5.1	0.0	0.0	0.0	7.9 ± 8.6	0.0
An. crucians/bradleyi	41/46	0.0	87.8 ± 10.0	0.0	4.9 ± 6.6	2.4 ± 4.7	0.0	0.0	0.0	4.9 ± 6.6	0.0
An. punctipennis	13/14	0.0	92.3	0.0	0.0	7.7	0.0	0.0	0.0	0.0	0.0
An. quadrimaculatus	382/405	0.0	91.1 ± 2.9	0.3 ± 0.5	3.9 ± 1.9	1.6 ± 1.2	0.5 ± 0.7	0.3 ± 0.5	0.3 ± 0.5	2.1 ± 1.4	0.0
Cs. melanura	6/7	0.0	50.0	0.0	0.0	33.3	0.0	0.0	0.0	16.7	0.0
Cx. pipiens	37/54	2.7 ± 5.2	16.2 ± 11.9	2.7 ± 5.2	21.6 ± 13.2	10.8 ± 10.0	0.0	8.1 ± 8.8	0.0	37.8 ± 15.6	0.0
Cx. restuans	7/8	0.0	14.3	0.0	14.3	14.3	0.0	42.9	0.0	14.3	0.0
Cx. salinarius	35/41	0.0	74.3 ± 14.5	2.9 ± 5.5	0.0	8.6 ± 9.3	0.0	0.0	0.0	14.3 ± 11.6	0.0
Oc. sollicitans	14/15	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oc. thibaulti	3/9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0
New York											
Ae. cinereus	27/30	0.0	85.2 ± 13.4	0.0	0.0	0.0	0.0	0.0	0.0	11.1 ± 11.9	3.7 ± 7.1
Ae. vexans	36/40	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cq. perturbans	21/28	0.0	85.7 ± 15.0	0.0	14.3 ± 15.0	0.0	0.0	0.0	0.0	0.0	0.0
Oc. canadensis	4/5	0.0	75.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0
Oc. japonicus	48/53	0.0	97.9 ± 4.0	0.0	2.1 ± 4.0	0.0	0.0	0.0	0.0	0.0	0.0
Oc. taeniorhynchus	7/8	0.0	85.7	0.0	0.0	14.3	0.0	0.0	0.0	0.0	0.0
Oc. triseriatus	27/31	0.0	88.9 ± 11.9	0.0	3.7 ± 7.1	7.4 ± 9.9	0.0	0.0	0.0	0.0	0.0
Oc. trivittatus	40/47	7.5 ± 8.2	90.0 ± 9.3	0.0	0.0	2.5 ± 4.8	0.0	0.0	0.0	0.0	0.0
Ps. ferox	5/5	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tennessee											
An. quadrimaculatus	151/163	0.7 ± 1.3	15.9 ± 5.8	76.2 ± 6.8	1.3 ± 1.8	2.0 ± 2.2	1.3 ± 1.8	2.0 ± 2.2	0.0	0.7 ± 1.3	0.0
Cx. erraticus	42/43	4.8 ± 6.4	11.9 ± 9.8	78.6 ± 12.4	2.4 ± 4.6	2.4 ± 4.6	0.0	0.0	0.0	0.0	0.0
Cx. pipiens complex	32/37	0.0	9.4 ± 10.1	53.1 ± 17.3	3.1 ± 6.0	6.3 ± 8.4	0.0	6.3 ± 8.4	0.0	18.8 ± 13.5	3.1 ± 6.0

[a]Only species for which the number of mammalian blood meals identified was three or more are shown. The first column indicates the number of mosquitoes identified and the overall number tested. All other columns indicate percentages (±95% CI where applicable) of meals from a given host species identified in a given mosquito species. 95% confidence intervals are provided for species where the number of samples identified was greater than or equal to 20.

TABLE 3. ANALYSIS OF AVIAN BLOOD MEAL SOURCES

	Percentage of meals taken from avian species[a]						
	New Jersey				*New York*		*Tennessee*
	Cs. melanura	Cx. pipiens	Cx. restuans	Cx. salinarius	Cx. pipiens	Cx. restuans	Cx. pipiens *complex*
Number identified/ number tested	44/61	23/43	7/11	5/10	9/10	6/7	36/67
American Robin	15.9 ± 10.8	30.4 ± 18.8	71.4	0.0	0.0	0.0	11.1 ± 10.3
Barn Swallow	0.0	0.0	0.0	20.0	0.0	0.0	0.0
Blue Jay	0.0	0.0	0.0	0.0	0.0	0.0	8.3 ± 9.0
Brown-headed Cowbird	0.0	13.0 ± 13.8	0.0	20.0	11.1	16.7	0.0
Carolina Chickadee	2.3 ± 4.4	0.0	0.0	0.0	0.0	0.0	0.0
American Crow	0.0	0.0	0.0	0.0	0.0	16.7	2.8 ± 5.4
Field Sparrow	0.0	4.3 ± 8.3	0.0	0.0	0.0	0.0	0.0
Green-backed Heron	0.0	0.0	0.0	0.0	0.0	16.7	0.0
Grey Catbird	4.5 ± 6.2	8.7 ± 11.5	0.0	0.0	0.0	0.0	11.1 ± 10.3
House Sparrow	0.0	0.0	0.0	0.0	0.0	0.0	2.8 ± 5.4
Northern Cardinal	27.3 ± 13.2	21.7 ± 16.9	0.0	20.0	0.0	0.0	19.4 ± 12.9
Northern Mockingbird	0.0	0.0	14.3	0.0	0.0	0.0	30.6 ± 15.0
Pine Warbler	4.5 ± 6.2	0.0	0.0	0.0	0.0	0.0	0.0
Red-winged Blackbird	4.5 ± 6.2	0.0	0.0	0.0	0.0	0.0	0.0
Ring-necked Pheasant	2.3 ± 4.4	8.7 ± 11.5	0.0	20.0	22.2	0.0	0.0
Scarlet Tanager	2.3 ± 4.4	0.0	0.0	0.0	0.0	0.0	0.0
Tufted Titmouse	25.0 ± 12.8	4.3 ± 8.3	14.3	0.0	33.3	33.3	0.0
Wood Thrush	0.0	0.0	0.0	0.0	22.2	16.7	0.0
Unknown A[b]	2.3 ± 4.4	0.0	0.0	0.0	0.0	0.0	0.0
Unknown B[c]	0.0	4.3 ± 8.3	0.0	0.0	0.0	0.0	13.9 ± 11.3
Unknown C[d]	2.3 ± 4.4	0.0	0.0	0.0	0.0	0.0	0.0
Unknown D[e]	4.5 ± 6.2	4.3 ± 8.3	0.0	20.0	0.0	0.0	0.0
Unknown E[f]	2.3 ± 4.4	0.0	0.0	0.0	0.0	0.0	0.0
Unknown F[g]	0.0	0.0	0.0	0.0	11.1	0.0	0.0

[a]Species for which three or more avian blood meals were identified are shown. The first row indicates the number of mosquitoes with avian derived blood meals identified and the overall number of mosquitoes with avian meals tested. All other rows indicate percentages (±95% CI where applicable) of avian derived meals from a given host identified in a given mosquito species. 95% confidence intervals are provided for those collections in which the number of individuals identified was equal to or greater than 20.

[b]Most similar to Brown-headed Cowbird (85% sequence identity).

[c]Most similar to British Jay (88% sequence identity).

[d]Most similar to Least Bittern (91% sequence identity).

[e]Most similar to European Robin (92% sequence identity).

[f]Most similar to Lark Sparrow (93% sequence identity).

[g]Most similar to Brown Thrasher (92% sequence identity).

host feeding patterns in 21 different mosquito species, 19 of which have been found to be infected with WNV. Although this represents the most comprehensive study of the host feeding patterns of mosquitoes infected with WNV reported to date, the 19 putative vector species examined represent less than half of the 43 North American mosquito species in which WNV infection has been documented. This study concentrated upon mosquitoes collected from natural and man made resting sites in primarily peri-urban areas. It is therefore possible that these sampling methods resulted in undersampling or exclusion of some important WNV vectors. More comprehensive studies employing a range of collection methods, and targeting ecologically diverse sites will be necessary to provide a completely comprehensive view of the host feeding patterns of the potential WNV vectors in the Eastern United States.

Although many of the species examined in this study have been reported to be infected with WNV, the vector competence for WNV of only a few of these mosquito species have been determined. *Ochlerotatus japonicus* was reported to be highly susceptible to infection,

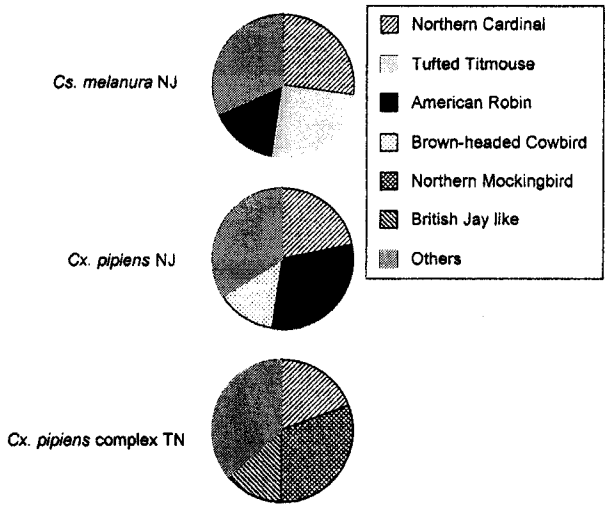

FIG. 1. Composition of avian derived blood meals from different collections. Data shown include only those collections (i.e., mosquitoes of a given species collected from a single location) in which the total number of avian derived blood meals was greater than 20.

while *Oc. sollicitans* was moderately susceptible (Sardelis and Turell 2001, Turell et al. 2001). In contrast, *Ae. vexans* and *Oc. taeniorhynchus* were relatively refractive to infection with WNV (Turell et al. 2000, 2001). Because of their mammalophilic feeding habits, these mosquito species could potentially serve as secondary vectors of WNV. *Culex restuans*, *Cx. salinarius* and members of the *Culex pipiens* complex (*Cx. pipiens* and *Cx. quinquefasciatus*) were reported to be highly to moderately competent to transmit WNV virus (Turell et al. 2000, 2001, Sardelis et al. 2001, Goddard et al. 2002). California populations of *Cx. quinquefasciatus* were reported to vary in susceptibility and, in general, be less susceptible to WNV infection than *Cx. pipiens.* (Goddard et al. 2002). The more susceptible populations of *Cx. quinquefasciatus* were collected in geographic locales where introgression of *Cx. pipiens* may have occurred. As previously suggested (Goddard et al. 2002), the consequence of this hybridization on vector competence for WNV requires further investigation. In our study, blood-fed mosquitoes were collected from areas of Tennessee where populations of *Cx. pipiens*, *Cx. quinquefasciatus* and hybrids of the two species are also sympatric. In our continuing investigation of the host-feeding habits of mosquitoes from Tennessee, we are incorporating molecular techniques (Aspen and Savage, 2003) to facilitate identification of members of *Cx. pipiens* complex, including hybrids, in part in an attempt to address this question.

In our study, mosquito species that fed most commonly on birds included the *Cx. pipiens* complex, *Cx. restuans*, and *Cs. melanura*. These results support previous studies that have reported these species to be primarily ornithophilic (Tempelis, 1975; Magnarelli, 1977; Nasci and Edman, 1981; Irby and Apperson, 1988; Apperson et al. 2002). Epidemic or bridge vectors of WNV display catholic host feeding habits that encompass both avian as well as mammalian hosts. *Culex salinarius* exhibited such a feeding pattern in both this study and in our previous study (Apperson et al. 2002). In the present investigation, mammalian blood meals were also identified in *Cx. pipiens* complex collected from Tennessee and *Cx. pipiens* from New Jersey. In view of these data, the involvement of the *Cx. pipiens* complex in the transmission of WNV in the U.S. as an epidemic as well as an enzootic vector appears probable.

In contrast, the *Anopheles*, *Aedes*, and *Ochlerotatus* species examined all fed primarily or exclusively on mammals. The description of the host feeding preferences of *An. quadrimaculatus s.s.* is notable, as this is the first report of the feeding habits of this species since the *An. quadrimaculatus* complex of sibling species was described (Reinert et al. 1997). These data corroborate previous studies (Apperson and Lanzaro, 1991; Robertson et al. 1993) reporting that *Anopheles* mosquitoes in this species complex fed mainly on large mammals. The discovery that *Oc. japonicus* fed primarily on white-tailed deer is also significant. There are no comparable studies of the feeding habits of *Oc. japonicus* from any other location, although Tanaka *et al.* [cited in (Sardelis and Turell, 2001)] observed natural populations of *Oc. japonicus* in Japan attacking humans and birds.

An interesting finding was that most of the mammalian blood meals identified in New York and New Jersey were derived from white-tailed deer, while those from Tennessee were primarily derived from dogs. Since this difference was consistent among the mosquito

TABLE 4. ABUNDANT BIRDS IN LOCATIONS WHERE MOSQUITOES WERE COLLECTED

Rank order	*New Jersey*[a]	*New York*[a]	*Tennessee*[a]
1	Laughing Gull	American Crow	Red-winged Blackbird
	Larus atricilla	*Corvus brachyrhyncho*	*Agelaius phoeniceus*
2	European Starling	American Robin	Common Grackle
	Sturnus vulgaris	*Turdus migratorius*	*Quiscalus quiscula*
3	Common Grackle	Common Grackle	European Starling
	Quiscalus quiscula	*Quiscalus quiscula*	*Sturnus vulgaris*
4	**American Robin**	Chipping Sparrow	Mourning Dove
	Turdus migratorius	*Spizella passerina*	*Zenaida macroura*
5	American Crow	Gray Catbird	**Northern Cardinal**
	Corvus brachyrhynchos	*Dumetella carolinensis*	***Cardinalis cardinalis***
6	Mourning Dove	House Finch	House Sparrow
	Zenaida macroura	*Carpodacus mexicanus*	*Passer domesticus*
7	House Sparrow	Song Sparrow	Eastern Meadowlark
	Passer domesticus	*Melospiza melodia*	*Sturnella magna*
8	Red-winged Blackbird	Northern Cardinal	American Robin
	Agelaius phoeniceus	*Cardinalis cardinalis*	*Turdus migratorius*
9	Northern Mockingbird	**Tufted Titmouse**	**Northern Mockingbird**
	Mimus polyglottos	***Baeolophus bicolor***	***Mimus polyglottos***
10	Gray Catbird	Black-capped Chickadee	Blue Jay
	Dumetella carolinensis	*Poecile atricapillus*	*Cyanocitta cristata*
11	House Finch	Mourning Dove	Northern Bobwhite
	Carpodacus mexicanus	*Zenaida macroura*	*Colinus virginianus*
12	Blue Jay	Blue Jay	Barn Swallow
	Cyanocitta cristata	*Cyanocitta cristata*	*Hirundo rustica*
13	Eastern Towhee	European Starling	Indigo Bunting
	Pipilo erythrophthalmus	*Sturnus vulgaris*	*Passerina cyanea*
14	**Northern Cardinal**	Canada Goose	Dickcissel
	Cardinalis cardinalis	*Branta canadensis*	*Spiza americana*
15	Rock Dove	House Wren	Chimney Swift
	Columba livia	*Troglodytes aedon*	*Chaetura pelagica*
16	**Tufted Titmouse**	House Sparrow	Red-bellied Woodpecker
	Baeolophus bicolor	*Passer domesticus*	*Melanerpes carolinus*
17	Barn Swallow	Red-eyed Vireo	American Crow
	Hirundo rustica	*Vireo olivaceus*	*Corvus brachyrhynchos*
18	Chipping Sparrow	Red-winged Blackbird	Common Yellowthroat
	Spizella passerina	*Agelaius phoeniceus*	*Geothylpis trichas*
19	Ovenbird	Baltimore Oriole	Rock Dove
	Seiurus aurocapillus	*Icterus galbula*	*Columba livia*
20	Chimney Swift	Brown-headed Cowbird	Killdeer
	Chaetura pelagica	*Molothrus ater*	*Charadrius vociferus*

[a]**Boldfaced** species were among the three most common avian blood meal hosts at each location.

species at each location, it is possible that this reflects variation in host availability in the northeastern states when compared to the peri-urban areas of Memphis, Tennessee. Alternatively, it is possible that differences in the choice of collection sites and/or the collection methods used in New York, New Jersey, and Tennessee may have in some way biased the collections towards acquisition of mosquitoes that fed upon different hosts in the two regions. Studies employing more carefully controlled choice of collection sites and identical collection methods in the two geographic areas will be necessary to resolve this question.

While blood meals from 24 avian species were detected, the majority of the blood meals taken at each of the three sites were derived from just three avian hosts. Generally, the most common avian hosts were abundant at each collection site. However, in many cases the proportion of blood meals taken from the most targeted species were greater than was predicted based upon their abundance. For example, the Northern Cardinal was host for 22.5 ± 8.4% of the avian blood meals identified in New Jersey, while representing just 1.5 ± 0.26% of the birds identified. Similar differences were noted for the Tufted Titmouse in New Jersey (16.2 ±

8.0% of the avian derived blood meals versus 1.4 ± 0.25% abundance) and the Northern Mockingbird in Tennessee (29 ± 14.4% of the avian derived blood meals versus 4.0 ± 0.64% abundance). Because the bird abundance data presented above is derived from the Breeding Bird Survey, and not from data collected at each collection point, it is possible that some of these differences reflect variations in the local abundance of the bird species in question, or in biases introduced by the mosquito collection methods used. However, given the study design, variations in local abundance are not likely to explain all of the differences seen. For example, the blood meal data from New Jersey were derived from collections from seven geographically separated sites, while the bird abundance data were collected from 10 separate survey routes. Among these 10 routes, the proportion of Northern Cardinals ranged from 0.9% to just 2.8%. Taken together, these data suggest certain of the more common bird species are fed upon more or less frequently than would be predicted based solely upon their local abundance. Differential feeding of mosquitoes on wild bird species is known to occur (Dow et al. 1957) and is caused in part by variation in the inherent attractiveness and anti-mosquito defensive behavior of avian species (Edman and Spielman 1988).

In the four summers since WNV was first detected in the United States, examination of dead American Crows for WNV has been found to be an effective surveillance strategy (Eidson et al. 2001, Centers for Disease Control and Prevention 2003a). In this regard, it is interesting to note that crow-derived blood meals were relatively rare in this study. This was despite the fact that the crow was the most abundant bird noted in Westchester County, New York, and was among the five most abundant species in New Jersey. This result is in concordance with recent work conducted at a site endemic for Eastern Equine Encephalomyelitis in Alabama, where American Crows were found to be significantly under-represented in the blood meals of the ornithophilic mosquitoes when compared to their observed abundance at the site (Hassan et al. 2003). In this regard, it is noteworthy that Komar and coworkers have documented contact transmission of WNV between crows (Komar et al. 2003). This observation brings into question whether the high level of crow mortality observed during WNV epizootics results from virus transmitted via mosquito bites or by bird-to-bird contact. More detailed studies will be necessary to clarify this question.

ACKNOWLEDGMENTS

We are grateful for the assistance of Stephanie Zellner and Todd Utz in completing blood meal analyses by ELISA. Thanks to Rebecca S. Levine for the careful analysis of many of the *Anopheles quadrimaculatus* samples and to the APHL/CDC Emerging Infectious Diseases Training Program for her support. We would also like to thank Louis Lacey, Director, Memphis-Shelby County Vector Control, for his support in the collection of mosquitoes from Tennessee. We thank M. Slamecka and J. Vellozzi for assistance with mosquito collections in New York. This research was supported in part by funds provided by the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention to CSA, TJD and TRU, and by a grant from the US National Institutes of Health/National Science Foundation program in the Ecology of Infectious Diseases (project R01-AI49724) to TRU.

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